EXAMINATION OF THE ACUTE AND SUBCHRONIC ORAL TOXICITY OF "COM KIEN TY" IN EXPERIMENTAL ANIMALS

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We evaluate the acute and subchronic toxicities of "Com kien ty" through oral administration in experimental animals. The acute toxicity was determined using the Litchfield Wilcoxon method in mice. Following WHO's recommendation, the subchronic toxicity was assessed in rabbits with oral doses of 0.9 g/kg/day (equal to the recommended human dose) and 2.7 g/kg/day (3 times as high as the recommended human dose) in 4 consecutive weeks. Results showed "Com kien ty" at the highest dose of 60.0 g/kg did not express acute toxicity in mice. Regarding the subchronic toxicity test, after oral administration of "Com kien ty", hematological parameters, hepato-renal functions were unchanged as compared with the control group, and no gross lesions in organs were observed in all experimental animals. In conclusion, "Com kien ty" did not produce acute and subchronic toxicities in Swiss mice and New Zealand rabbits.

Keywords: "Com kien ty", acute toxicity, subchronic toxicity, experimental animals.

I. INTRODUCTION

In modern times, the usage of medicinal plants and herbal medicines in therapy has been increasingly popular. The World Health Organization estimates that 80% of the world's population uses herbal medicinal products for their therapeutic virtues.[1] Herbal medicine has always represented an important component of primary health care. In recent years, herbal remedies have been considered as dietary supplements for disease prevention as well as alternative/complementary medicine. A wide variety of herbal medicines are readily available in the market all over the world. With the rising utilization of herbal products, the safety and efficacy of herbal medicine have become a public health concern.[2]

"Com kien ty" was a product of the Department of Pharmacy, Military Institute of Traditional Medicine. "Com kien ty" contained natural materials including *Poria cocos* Wolf., *Panax Ginseng*, *Magnolia officinalis* Rehd. et Wils., *Atractylodes lancea* (Thunb.) DC., *Pericarpium Citri Reticulatae, Amomum aromaticum* Roxb., and *Glycyrrhiza uralensis*. Historically, these herbs have been used in healthcare since ancient time and in folklore to treat many diseases and illnesses.[1]

"Com kien ty" was created for the purpose of supporting the treatment of gastrointestinal disorders. Following previous studies, *Panax Ginseng* was proven to exert positive effects on gastrointestinal diseases such as inflammatory bowel disease[3], irritable bowel syndrome[4],… Ethnopharmacological relevance *Poria cocos Wolf* has been used in traditional East-Asian medicine for centuries to effectively treat various gastrointestinal disorders such as diarrhea for its tonic, anti-fungal, and anti-bacterial activities.[5] Moreover, the plants of the genus *Amomum* are popularly used for the treatment of stomach diseases and digestive disorders.[6]

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In order to use "Com kien ty" in clinical, first and foremost, this product was required to be tested in preclinical research. In reality, so far, there have been no report available on the safety of a combination product from these components. Therefore, we conducted this study to investigate the acute and subchronic toxicities of "Com kien ty" in animals.

II. MATERIALS AND METHODS

1. The preparation of "Com kien ty"

"Com kien ty" was manufactured by the Department of Pharmacy, Military Institute of Traditional Medicine. The product achieved the Standard Basis from The National Institute of Drug Quality Control. "Com kien ty" was formulated in form of sachets of granules, and every 01 sachet contained 05 g dry extract from natural materials including *Poria cocos* Wolf., *Panax Ginseng*, *Magnolia officinalis* Rehd. et Wils., *Atractylodes lancea* (Thunb.) DC., *Pericarpium Citri Reticulatae, Amomum aromaticum* Roxb., and *Glycyrrhiza uralensis*. These materials were prepared, extracted, concentrated, dried, grinded, mixed with *Lactobacillus acidophilus*, and granulated. The human recommended dose was 03 sachets daily.

2. Experimental animals

Swiss mice (18 – 20 g) and *New Zealand* rabbits (2.0 –2.2 kg) were supplied by National Institute of Hygiene and Epidemiology. The animals were housed in cages with access to a standard certified rodent diet and water ad libitum. They were acclimated to housing for 5 – 7 days before the experiment at the National Institute of Drug Quality Control.

3. Acute toxicity study

Acute toxicity study was conducted according to WHO Guidance. [7]

Mice were fasted for 15h before the experiment and had water ad libitum. Mice were orally administered with "Com kien ty" through feeding needles. Mice were divided into 4 groups including control group administered distilled water and 3 groups "Com kien ty" with the planned doses: 20.0 g/kg b.w, 40.0 g/kg b.w, and 60.0 g/kg b.w;

The general symptoms of toxicity and mortality in each group were observed within 24 hours, 72 hours and 7 days after administration. Based on the rate of animal deaths within 24 hours, the median lethal dose (LD50) was calculated by the Litchfield-Wilcoxon method.[8]

4. Subchronic toxicity study

Subchronic toxicity study was carried out according to WHO Guidance.[7]

The study was carried out in the course of continuous four weeks. Rabbits were divided into three groups of seven animals:

- Group 1 (control group) was given an administration of distilled water;

- Group 2 was administered orally "Com kien ty" at the dose of 0.9 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3 was administered orally "Com kien ty" at the dose of 2.7 g/kg/day (3 times as high as the dose at group 2).

Animals were given the oral administration of distilled water and "Com kien ty" with the volume 5 mL/kg b.w daily for consecutive four weeks and observed once daily to detect clinical signs and time points for laboratory tests.

The signs and parameters were checked during the study, including general condition, mortality, and clinical signs.

- Bodyweight changes

- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), platelet count (PLT).

- Serum biochemistry test: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, urea, and creatinine levels.

The parameters were checked before treatment and four weeks after treatment.

- After 4 weeks of treatment, all rabbits were subjected to a full gross necrospy.

4. Statistical analysis

Data were analyzed using SPSS version 13.0. The significance levels between the experimental groups and the control group were made using the student's t-test and Avant-après test. Data were shown as mean ± standard deviation. All data were considered significant at $p < 0.05$.

III. RESULTS

1. Acute toxicity study

In the oral acute toxicity test, "Com kien ty" treated animals showed no mortality at ascending doses from 20 g/kg to 60.0 g/kg body weight within 72 h and for additional seven days. There was no toxicity sign such as piloerection, lacrimation, or changes in locomotion and respiration.

2. Subchronic toxicity study

General condition

Animals had normal locomotor activities and good feedings. None of the treated animals in all groups showed any macroscopic or gross pathological changes t compared with the control group.

Body weight changes

Figure 1 showed that after 4 weeks, the body weight at all groups increased slightly as compared with the time point "Before treatment". After 4 weeks of treatment, at group 1, there was a significant increase in the body weight as compared with the time point "Before treatment" ($p < 0.01$).

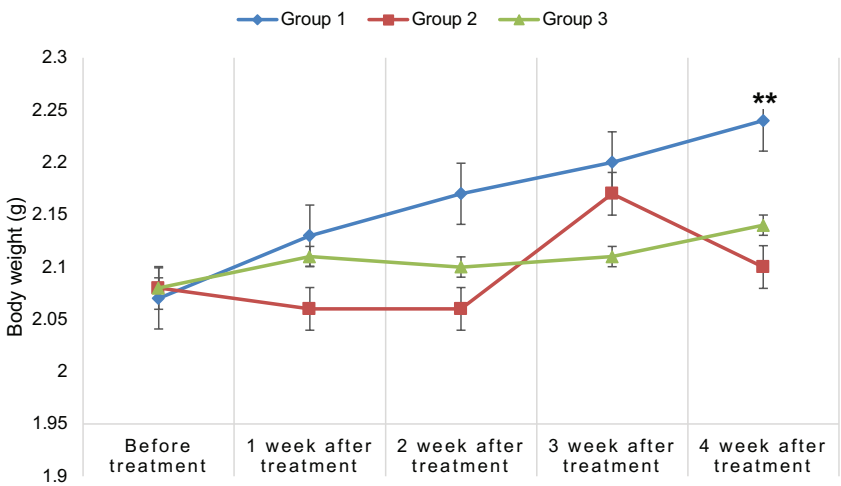

Figure 1. The effect of "Com kien ty" on body weight changes

*** p < 0.01 as compared with the time point "Before treatment"*

3. The effect of "Com kien ty" on the hematological system

There were no significant difference in red blood cell count, hematocrit, hemoglobin level, platelet count and total WBC count between "Com kien ty" treated groups and control group ($p > 0.05$) (Table 1).

Table 1. The effect of "Com kien ty" on hematopoietic function

Parameters	Group	Before treatment	After 4 weeks of treatment
Red blood cells count (T/L)	Group 1	5.5 ± 0.3	5.9 ± 0.4
	Group 2	5.5 ± 0.2	5.7 ± 0.4
	Group 3	5.4 ± 0.4	6.0 ± 0.4
	p	> 0.05	> 0.05
Hemoglobin level (g/dL)	Group 1	10.8 ± 0.5	11.0 ± 0.5
	Group 2	11.1 ± 0.5	11.2 ± 0.4
	Group 3	10.8 ± 0.8	11.4 ± 0.5
	p	> 0.05	> 0.05
Hematocrit (%)	Group 1	35.0 ± 1.5	38.0 ± 1.7
	Group 2	36.0 ± 1.8	37.0 ± 1.7
	Group 3	35.2 ± 2.9	38.0 ± 2.0
	p	> 0.05	> 0.05
Total WBC count (G/L)	Group 1	8.4 ± 1.5	8.0 ± 1.4
	Group 2	8.6 ± 0.9	7.9 ± 1.0
	Group 3	8.1 ± 1.7	8.3 ± 2.1
	p	> 0.05	> 0.05
Platelet count (G/L)	Group 1	474.9 ± 52.1	554.7 ± 56.7
	Group 2	450.0 ± 53.2	563.7 ± 100.3
	Group 3	448.6 ± 94.6	522.0 ± 117.8
	p	> 0.05	> 0.05

*p: compared with the control group and the time point "**"Before treatment"*

4. The effect of "Com kien ty" on liver functions

There were no significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT) level and total bilirubin concentration between "Com kien ty" treated groups and the control group (p > 0.05). The results are shown in Table 2.

Table 2. The effect of "Com kien ty" on liver functions

Parameters	Group	Before treatment	After treatment	
			Two weeks	Four weeks
AST level (UI/L)	Group 1	73.50 ± 10.06	69.40 ± 9.37	73.20 ± 13.88
	Group 2	73.90 ± 8.41	78.60 ± 11.68	69.00 ± 10.85
	Group 3	66.90 ± 12.26	73.40 ± 14.45	79.10 ± 14.17
	p	> 0.05	> 0.05	> 0.05
ALT level (UI/L)	Group 1	27.30 ± 5.25	32.70 ± 5.68	27.20 ± 2.66
	Group 2	30.70 ± 6.13	34.80 ± 8.78	29.40 ± 7.55
	Group 3	31.10 ± 6.17	34.30 ± 6.36	31.50 ± 7.41
	p	> 0.05	> 0.05	> 0.05
Total bilirubin (mmol/L)	Group 1	10.15 ± 0.78	9.68 ± 0.91	9.70 ± 0.72
	Group 2	9.96 ± 0.91	9.72 ± 1.19	9.46 ± 0.99
	Group 3	9.70 ± 0.72	9.40 ± 0.91	9.88 ± 1.02
	p	> 0.05	> 0.05	> 0.05

p: compared with the control group and the time point "Before treatment"

5. The effect of "Com kien ty" on kidney functions

Figure 2 demonstrated that after four weeks of treatment, "Com kien ty" caused no significant difference in serum urea and creatinine level between the control group and the two treated groups (p > 0.05).

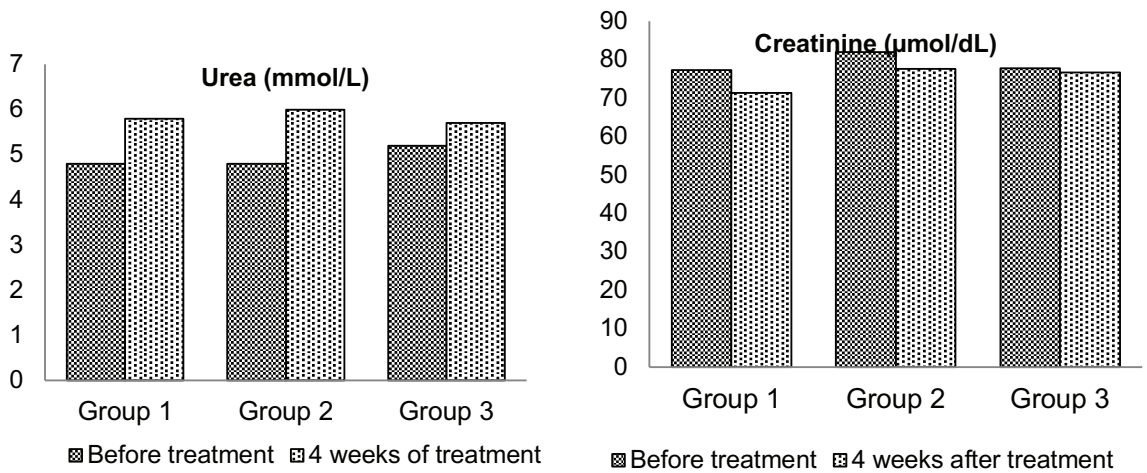

Figure 2. The effects of "Com kien ty" on kidney functions

6. The effect of "Com kien ty" on macroscopic examination

No gross lesion in the heart, lungs, livers, spleen, pancreas, kidneys or digestive system were observed in all experimental rabbits (control group and 2 groups treated "Com kien ty).

IV. DISCUSSION

1. Acute toxicity of "Com kien ty"

In this experiment, the acute oral toxicity test showed that "Com kien ty" was tolerated up to 60.0 g/kg (approximately 16.7 times as high as recommended human dose). Moreover, no sign of toxicity and no mortality were observed for continuous seven days. As a result, oral LD50 of "Com kien ty" were not determined in mice. As defined by WHO, "Com kien ty" was a safe herbal medicine.

2. Subchronic toxicity of "Com kien ty"

Toxicity is the degree to which a substance can harm humans or animals. Toxicity can refer to the effect on a cell (cytotoxicity), an organ (e.g., renal or liver toxicity), or the whole organism.[8] To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animal models to detect toxicity and provide guidelines for selecting 'safe' therapeutic doses in human. A subchronic toxicity study provided information on the effects of repeated oral exposure and indicated the need for longer-term studies.[9] Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of animals' average life span, such as rodents. Specifically, they provide information on target organ toxicity.[10]

The body weight changes are the most basic index to reflect toxicity to organs and systems and reflect the combined effects of xenobiotics on the body.[10] For all experimental animals, general signs should be observed daily, and body weight should be measured periodically.[9] It can be stated that *"*Com kien ty*"* did not interfere with animals' normal metabolism as corroborated by the non-significant difference from animals using the distilled water as the control group.

The blood circulatory system performs essential functions, for example, delivering oxygen to all body tissues, maintaining vascular integrity, providing necessary immune factors for host defense reaction, and so on. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an essential parameter for humans and animals' physiological and pathological status.[9] Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies. After two weeks and four weeks of treatment, there was no significant difference in total red blood cells, hematocrit, hemoglobin level, platelet count and total WBC count between the "Com kien ty" treated groups and the control group, so it can be concluded that the "Com kien ty" do not affect the hematological system.

Analysis of kidney and liver is critical in the toxicity evaluation of drugs and plant extracts as they are both necessary for an organism's survival. The clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.[11] The changes of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) contents is a sensitive index to reflect the degree of liver cell damage. When there is chronic liver injury, AST and ALT would be released from the liver cells' injury, and the serum level is increased.[8] Urea

and creatinine levels can be used in describing the function of the kidneys.[9] There are no significant AST, ALT, urea, creatinine changes in rabbits at all doses, which indicates that "Com kien ty" had no deleterious effect on liver and kidney functions. The blood biochemistry level of control and "Com kien ty" in treated rabbits at various doses are presented no significant difference between "Com kien ty" treated groups and the control group ($p > 0.05$). This evidence shows that "Com kien ty" did not affect the liver and kidney functions. Moreover, no gross lesion was observed at a full gross necropsy which examined of the hearts, lungs, livers, spleens, pancreas, kidneys or digestive system.Overall, this study's findings indicated that no significant difference was observed in blood parameters and biochemistry parameters between the "Com kien ty" treated groups and the control group. Results of our study were consistent with previous reports about the toxicity of components in "Com kien ty". Administration of Panax ginseng extract for 13 consecutive weeks did not result in any toxicologically significant changes in mortality, body weight, hematology, serum biochemistry, and gross pathological findings.[12] In the subchronic toxicity test, after 4 weeks of treatment of a medical formula with the main active component (*Pericarpium Citri Reticulatae*), the overall health, body weights, blood parameters, and biochemistry indexes of animals did not show any change as compared with control group.[13]

V. CONCLUSION

No signs of toxicity and no mortality were observed in "Com kien ty" treated mice at the dose of 60.0 g/kg (approximately 16.7 times as high as recommended human dose). Oral LD_{50} of "Com kien ty" were not determined in mice.

"Com kien ty" at oral doses of 0.9 g/kg/day and 2.7 g/kg/day administered at 4 continuous weeks did not yield any toxic sign or symptom of subchronic toxicities in rabbits.

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